

**Claims**

1. An assay for primary HIV comprising:  
a cell line that expresses CCR5, CXCR4 and CD4 receptors and a marker gene, said receptors adapted to bind and facilitate entry of said primary HIV into cells at said cell line,  
wherein marker gene expression indicates HIV infection.
2. The assay of claim 1 wherein said cell line is immortal.
3. The assay of claim 1 wherein said cell line originates from HeLa.
4. The assay of claim 1 wherein said marker gene encodes luciferase.
5. The assay of claim 1 wherein said marker gene encodes a fluorescence protein.
6. The assay of claim 1 further comprising an amplicon gene, wherein the expression of said amplicon gene increases primary HIV production.
7. The assay of claim 6 wherein said amplicon gene facilitates the production of drug resistant minor species of primary HIV.
8. The assay of claim 6 wherein said amplicon gene is Tat.

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1           9.     The assay of claim 1 wherein marker gene expression is in near  
2 linear quantities to HIV infection of cells of said cell line over at least two orders  
3 of magnitude.

1           10.    The assay of claim 1 wherein said immortalized cell line is J53tat.

1           11.    The assay of claim 1 wherein said immortalized cell line is J53BL.

1           12.    The assay of claim 1 for the measurement of HIV attributes  
2 selected from the group consisting of: co-receptor utilization, drug sensitivity,  
3 antibody neutralization, isolation and titration.

1           13.    A composition comprising: an immortalized cell line having  
2 receptors for binding a primary HIV and having a marker gene expressed in  
3 response to binding of HIV, wherein marker gene expression correlates to the  
4 magnitude of virus binding to said receptors and entry into cells of said cell line.

1           14.    The composition of claim 13 further comprising primary HIV  
2 within cells of said cell line.

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1 15. The composition of claim 13 wherein said immunodeficiency virus  
2 is a primary virus, said primary virus being amplified in less than three days to  
3 create a virus stock.

1 16. The composition of claim 13 wherein said immortalized cell line  
2 is J53tat and functional equivalents thereof.

1 17. The composition of claim 13 wherein said immortalized cell line  
2 is J53BL and functional equivalents thereof.

1 18. A method for producing primary HIV infection sensitive clonal  
2 cells, comprising the steps of:  
3 selecting a cell line expressing CCR5, CXCR4 and CD4 receptors;  
4 transducing said cell line with a gene vector encoding for luciferase such  
5 that luciferase expression correlates to the magnitude of HIV infection of said cell  
6 line; and  
7 establishing said clonal cells.

1 19. A method of determining a primary HIV titer, comprising:  
2 infecting a cell line with a quantity of primary HIV, wherein said cell line  
3 expresses a marker gene product, CCR5, CXCR4 and CD4 receptors, wherein the  
4 marker gene product expression increases in response to said cell line infection  
5 with said quantity of HIV;

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6 allowing sufficient time for said quantity of HIV to bind and enter into said  
7 cell line; and  
8 measuring the marker gene expression.

1 20. A method of determining primary HIV phenotypic drug sensitivity,  
2 comprising the steps of:  
3 infecting a cell line with a quantity of primary HIV in the presence of a  
4 drug candidate; wherein said cell line expresses a marker gene product, CCR5,  
5 CXCR4 and CD4 receptors, wherein the marker gene product expression increases  
6 in response to said cell line infection with said quantity of HIV;  
7 allowing sufficient time for said primary HIV to bind and enter said cell  
8 line; and  
9 measuring the marker gene product in response to said quantity of primary  
10 HIV.

1 21. The method of claim 20 further comprising the step of infecting a  
2 second immortalized cell line with a quantity of HIV wherein said second cell line  
3 expresses an amplicon gene, CCR5, CXCR4 and CD4 receptors, wherein  
4 amplicon expression increases said quantity of HIV.

1 22. A method of amplifying primary HIV to create a virus stock  
2 comprising the steps of:

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3 infecting a cell line with a quantity of primary HIV; wherein said cell line  
4 expresses an amplicon gene, CCR5, CXCR4 and CD4 receptors, wherein the  
5 amplicon gene expression increases in response to said cell line infection with said  
6 quantity of HIV;

7 allowing sufficient time for primary HIV to bind, enter and replicate in  
8 said cell line to form said virus stock; and

9 isolating said virus stock.

1 23. The method of claim 22 further comprising the steps of:


2 infecting a second cell line with a quantity of primary HIV in the presence  
3 of a drug candidate; wherein said second cell line expresses a marker gene  
4 product, CCR5, CXCR4 and CD4 receptors, wherein the marker gene product  
5 expression increases in response to said second cell line infection with said  
6 quantity of HIV;

7 allowing sufficient time for said primary HIV to bind and enter said second  
8 cell line; and

9 measuring the marker gene product in response to said quantity of primary  
10 HIV.

1 24. The method of claims 19, 20 or 22 wherein said primary HIV is

2 HIV-1.



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1            25.    The method of claims 19 or 20 wherein said immortalized cell line  
2            is J53BL.

1           26.     The method of claims 19 or 20 wherein said cell line expresses an  
2     amplicon.

1            27.    The method of claim 26 wherein said amplicon is Tat.

28. The method of claims 19, 20 or 23 wherein the marker gene product is selected from the group consisting of: luciferase,  $\beta$ -galactosidase, green fluorescent protein and chloramphenicol acetyltransferase.

1            29.     The method of claims 19, 20 or 22 wherein infecting said cell line  
2     with said quantity of immunodeficiency virus occurs in the presence of an  
3     infectivity complement.

1           30. The method of claim 29 wherein said infectivity complement is  
2           selected from a group consisting of VSV-G, adenovirus, liposome and monoclonal  
3           antibody.

1                    31.     The method of claims 18, 19, 20 or 22 wherein said quantity of  
2     virus is derived from blood plasma.

1           32.     The method of claim 22 wherein said quantity of HIV comprises  
2     a major population and a minor population having a number ratio therebetween  
3     and the minor population is amplified to a greater extent than the major population  
4     so as to change the number ratio.

1           33.     The method of claims 18, 19, 20 or 22 wherein said quantity of  
2     primary HIV is derived from cell culture.

1           34.     The use of an infectivity complement in conjunction with the  
2     composition of claim 13.

1           35.     The use of an infectivity complement in conjunction with the  
2     method of claims 18, 19, 20 or 22.

1           36.     A cell-based assay according to claim 1 substantially as described  
2     herein in any of the examples.

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1. A cell for amplifying virus gene expression, said cell comprising expressed CCR5, CXCR4, and CD4, and further comprising an expressible nucleic acid sequence encoding an amplicon.

2. The cell of claim 1, wherein said cell is part of an assay system further comprising:

an indicator cell, said indicator cell comprising expressed CCR5, CXCR4, and CD4, and a marker gene, said marker gene responsive to the presence of virus.

3. The cell or assay system of claim 1 or 2 wherein said virus is primary HIV and wherein said amplicon is Tat.

4. The assay system of claim 2 wherein said marker gene is selected from the group of marker genes consisting of  $\beta$ -galactosidase, luciferase, fluorescent protein, and antibiotic resistance.

5. The cell or assay system of claim 1 or 2 wherein said expressible nucleic acid sequence encoding said amplicon is driven by a promoter selected from the group of promoters consisting of constitutive and inducible promoters.

6. The cell or assay system of claim 5 wherein said promoter is selected from the group of promoters consisting of CMV and Tet.

7. The cell or assay system of claim 1 or 2 wherein said cell is infected with said virus and cultured in the presence of an antiviral drug to detect virus susceptibility to said drug.

8. An assay system comprising the cell of claim 1 or 2 wherein virus produced in said cell is subsequently detected using an antibody.



9. The assay system of claim 8 wherein said antibody is used in an ELISA assay.

10. A method of propagating a virus comprising:  
5 providing a cell with a virus to be isolated, wherein said cell expresses an amplicon for amplifying said virus; and  
amplifying said virus in said cell using said amplicon.

11. The method of claim 10 further comprising  
10 detecting said amplified virus using one or more of an antibody assay or an indicator cell, said indicator cell infectable with said amplified virus and comprising a marker gene responsive to the presence of said virus to thereby enable detection of said virus.

12. The method of claim 10 or 11 wherein said cells for amplifying said virus and detecting said virus each express CCR5, CXCR4, and CD4.

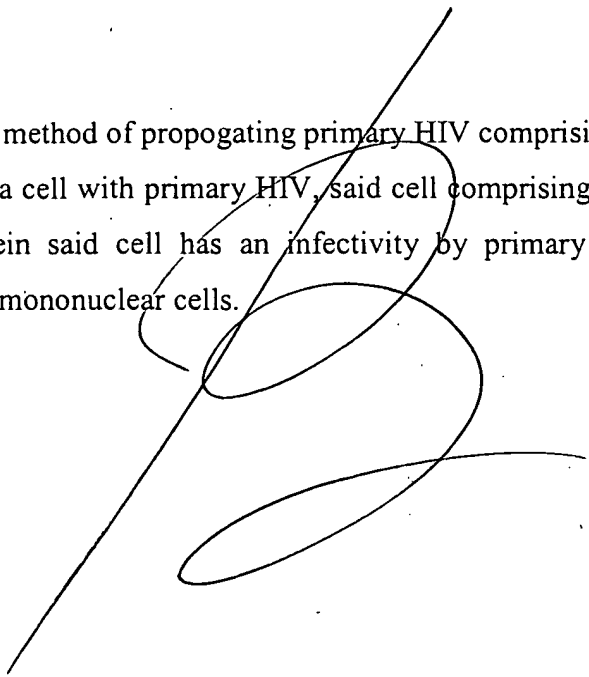
13. The method of claim 12 wherein said virus is a drug-resistant virus and wherein said amplifying is conducted in the presence of a drug to which said virus is  
20 resistant.

14. The method of claim 13 wherein said virus is a primary HIV virus and wherein said amplicon is Tat.

15. The method of claim 13 wherein said marker gene encodes a member  
25 selected from the group consisting of: luciferase,  $\beta$ -galactosidase, green fluorescent protein and antibiotic resistance.

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16. A method of propagating primary HIV comprising:  
infecting a cell with primary HIV, said cell comprising expressed CCR5, CXCR4,  
and CD4, wherein said cell has an infectivity by primary HIV greater than that of  
peripheral blood mononuclear cells.



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